

FIG. 1

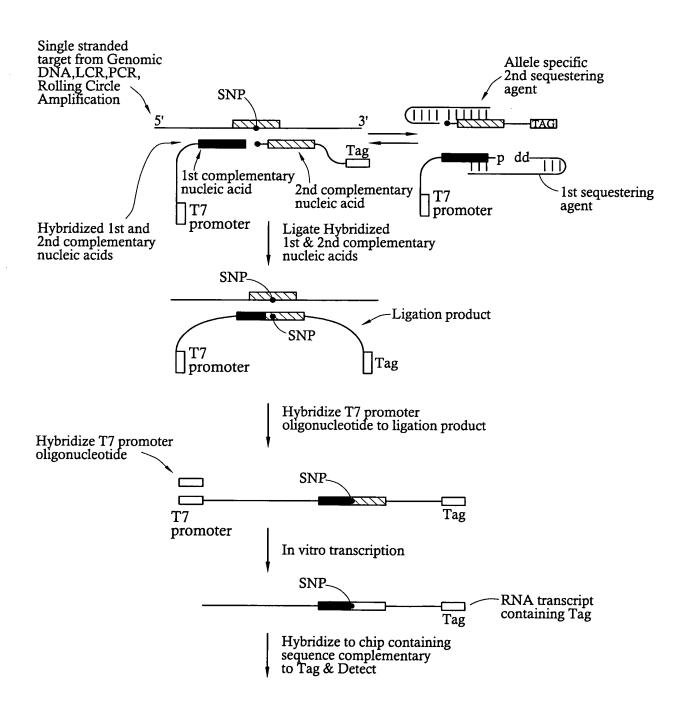


FIG. 2

OLIGONUCLEOTIDE SEQUESTERING AGENTS AND METHODS OF USE Donald M. Crothers

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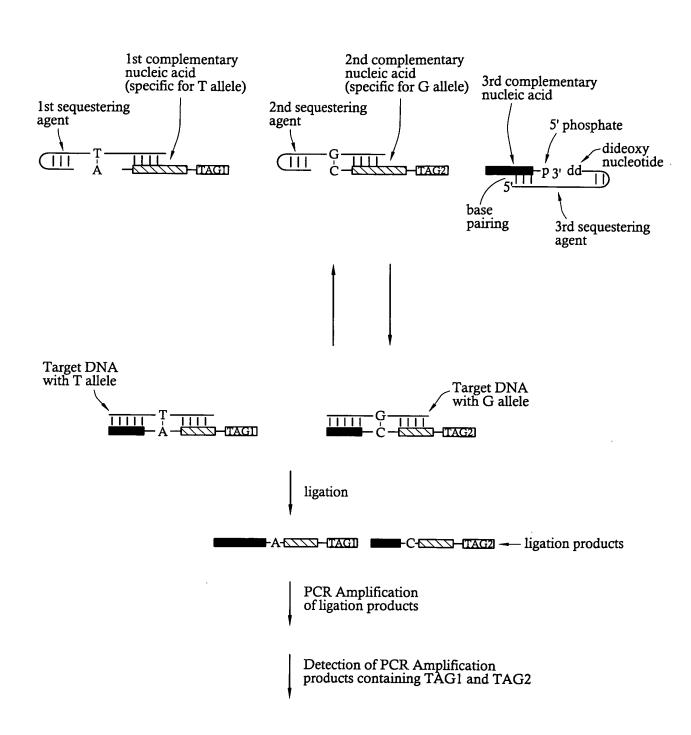


FIG. 3

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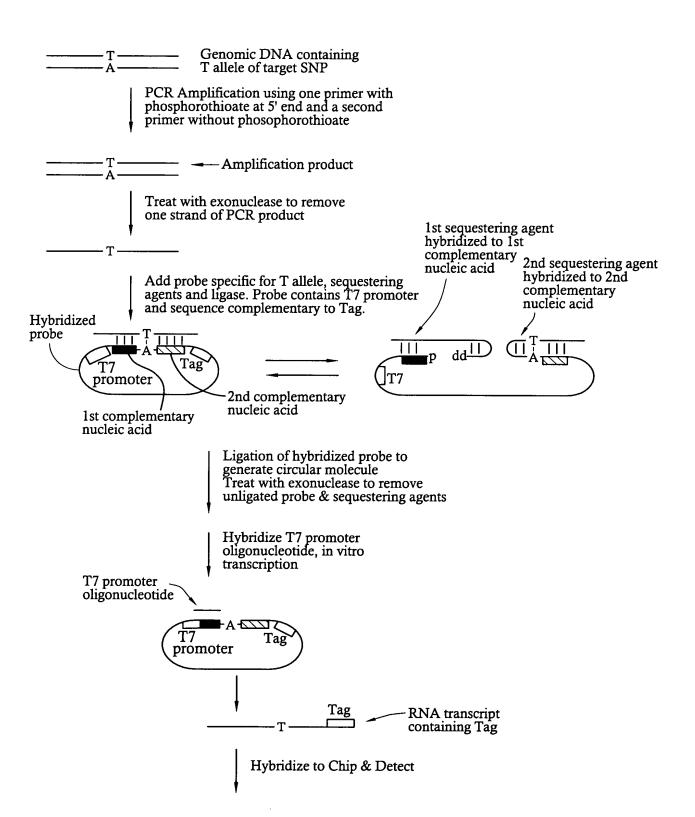


FIG. 4

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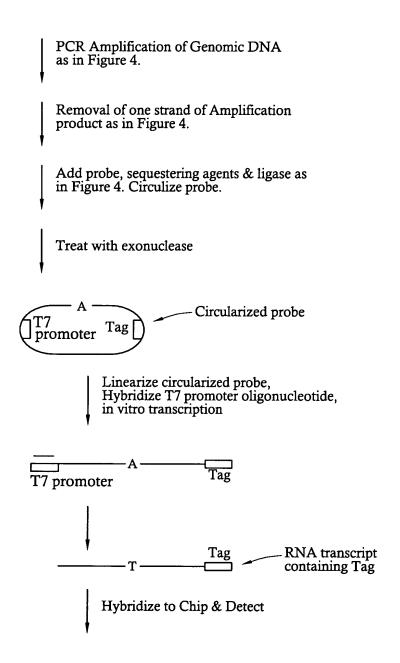


FIG. 5

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PCR Amplification of Genomic DNA as in Figure 4.

Removal of one strand of Amplification product as in Figure 4.

Add probe, sequestering agents & ligase as in Figure 4. Circulize probe. Probe does not contain T7 promoter.

Treat with exonuclease as in Figure 4.

Linearize circularized probe

Tag

Hybridize to Chip & Detect

FIG. 6

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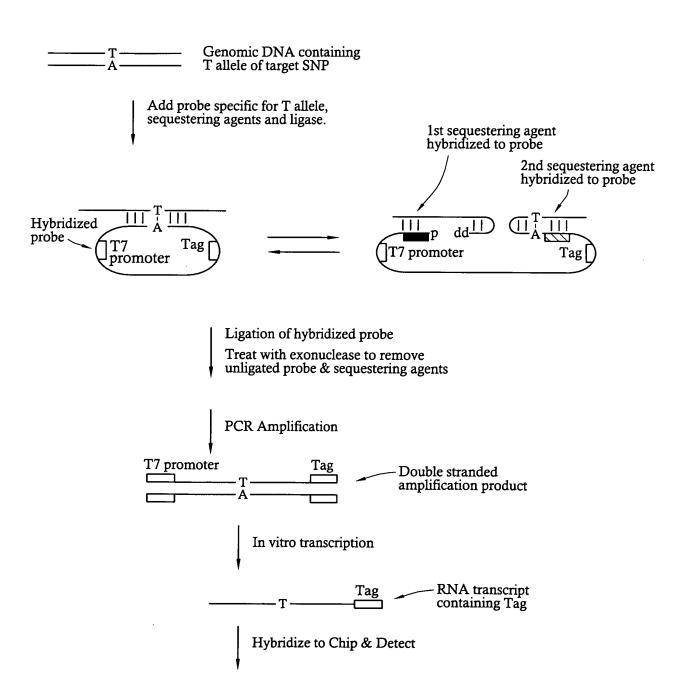


FIG. 7

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Add probe, sequestering agents & ligase as in Figure 7. Circulize probe. Probe does not contain T7 promoter.

Treat with exonuclease as in Figure 7.

PCR Amplification as in Figure 7.

Tag Double stranded amplification product

Hybridize to Chip & Detect

FIG. 8

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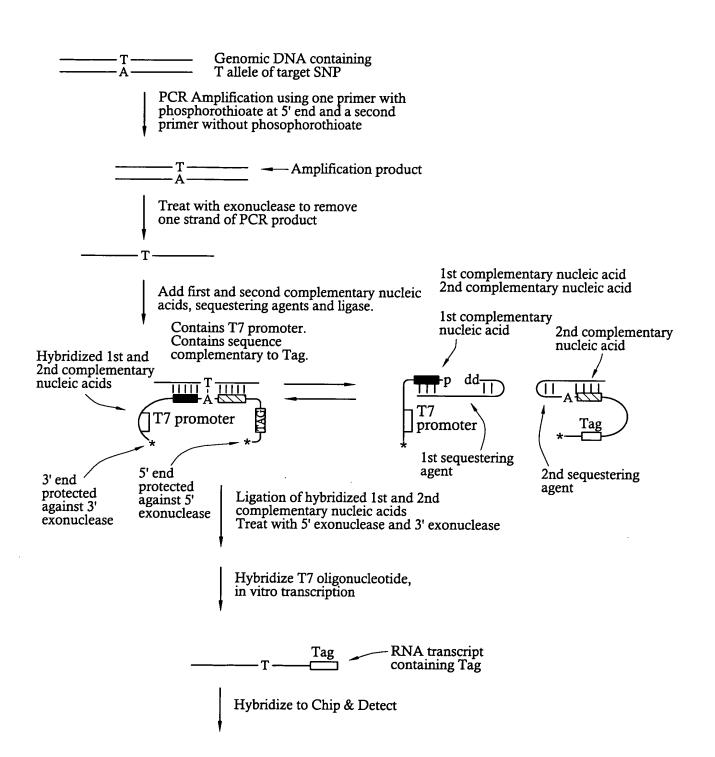


FIG. 9

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PCR Amplification using one primer with phosphorothioate at 5' end and a second primer without phosophorothioate as in Figure 9.

Treat with exonuclease to remove one strand of PCR product as in Figure 9.

Add first and second complementary nucleic acids, sequestering agents and ligase as in Figure 9. 1st complementary nucleic acid does not contain T7 promoter.

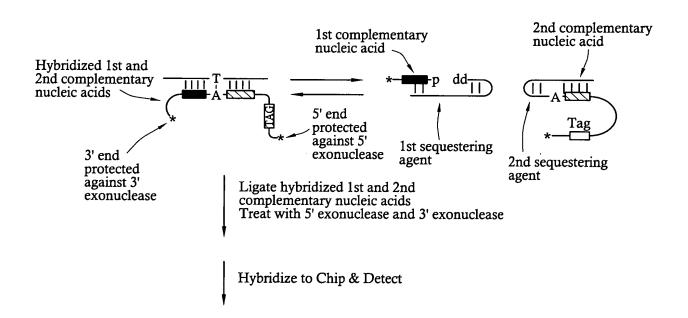
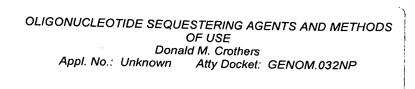


FIG. 10



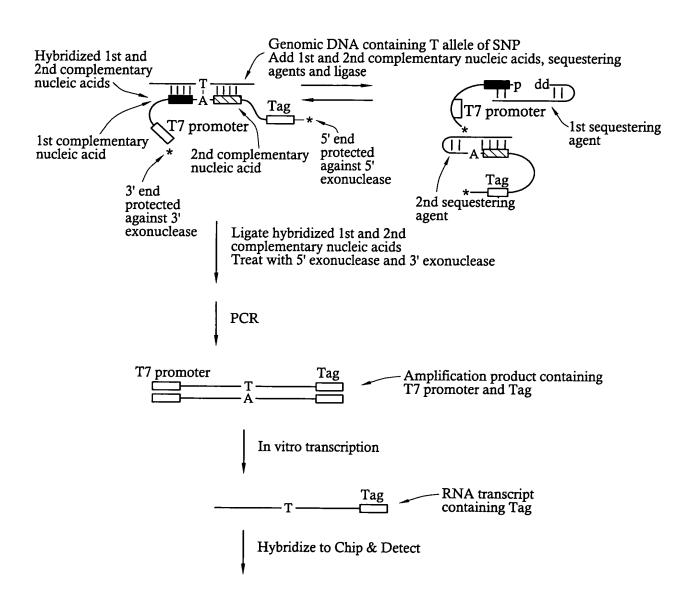


FIG. 11

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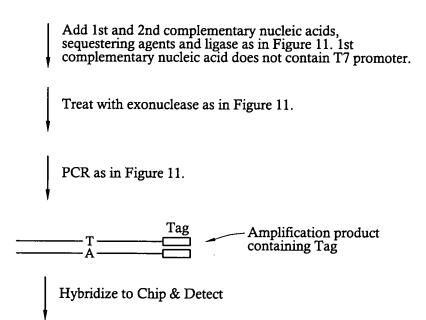


FIG. 12

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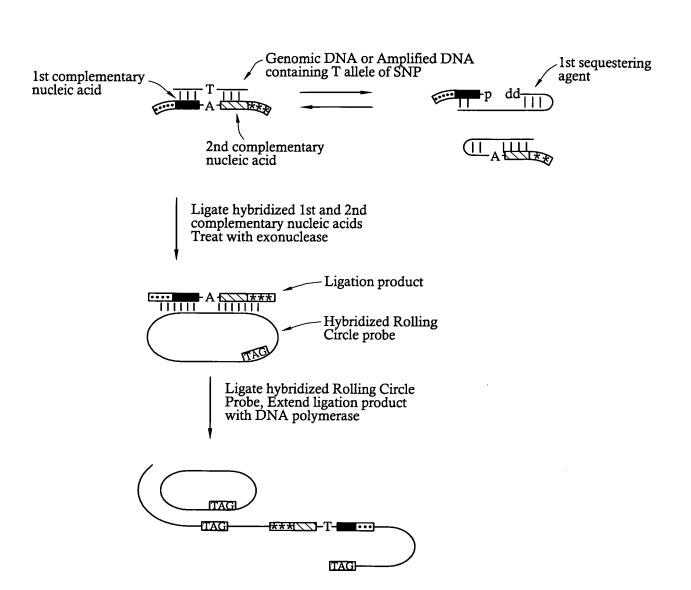


FIG. 13

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SNP base

(SEQ ID NO:20) 5' -CTAATCTGTAAGAGCAGATCCCTGGACAGGC AAG. (F5 1698 T7 Probe) ... AGACATTCTCGTCTAGGGACCTGTCCG-P (SEQ ID NO:2)

Calculated Tm = 87.1

(SEQ ID NO:2) 3' -AGACATTCTCGTCTAGGGACCTGTCCG-P ddGCCTTCGCCGTTT

(SEQ ID NO:5) (5') GATCCCTGGACAGGC----CGGAAGCGGCTTT

Hybrid Tm = 77.2

Hairpin Tm = 81.0

Illustration of a suppressor of non-specific ligation: Sequestering agent for the 5'side ligation probe for Factor V

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SNP base 5'-AAGGAATACAGGTATTTTGTCC-3'... (SEQ ID NO:2) (F5 1698 Tag Probe).OH-TTCCTTATGTCCATAAAACAGG (SEQ ID NO:3)

Calculated Tm = 75.4

3'- TTTGCAGAGCCGTG-5' OH-TTCCTTATGTCCATAAAACAGG (SEQ ID NO:3)

TTTCGTCTCGGCAC-----AAGGAATACAGGT (SEQ ID NO:5)

Hairpin Tm = 81.0

Hybrid Tm = 64.2

Illustration of a suppressor of non-specific ligation: Sequestering agent for the 3'-side ligation probe for Factor V

SNP base

(SEQ ID NO.20) 5' - CTAATCTGTAAGAGCAGATCCCTGGACAGGC AAG.

TAGGGACCTGTCCG-P-5' (F5 1698 T7 Probe) ..-

(SEQ ID NO:22)

Calculated Tm = 75.4

(SEQ ID NO:22) 3' -TAGGGACCTGTCCG-P DD-GCCTTCGCCGTTT

CCTGGACAGGC----CGGAAGCGGCTTT (SEQ ID NO:23) (5')

Hybrid Tm = 67.7

Hairpin Tm = 81.0

Illustration of a suppressor of non-specific ligation: Sequestering agent for the 5'side ligation probe for Factor V

FIG. 16A

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base SNP

(SEQ ID NO:24) 5'-AAGGAATACAGGTATTTTGTCCTTG-3'. (F5 1698 Tag Probe).OH-TTCCTTATGTCCATAAAACAGGAAC-5'

(SEQ ID NO:25)

Calculated Tm = 77.8

TTTGCAGAGCCGTG-5' OH-TTCCTTATGTCCATAAAACAGGAAC-5' (SEQ ID NO:25)

TTTCGTCTCGGCAC-----AAGGAATACAGG (SEQ ID NO:26)

Hairpin Tm = 81.0

Hybrid Tm = 69.7

Illustration of a suppressor of non-specific ligation: Sequestering agent for the 3'-side ligation probe for Factor V